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LIGHT COLLECTION AND EXCITON DYNAMICS IN PHOTOSYNTHETIC MEMBRANES*

by

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ABSTRACT

The structural organization for the light collection apparatus in higher plants is investigated by means of picosecond fluorescence spectroscopy. The exciton dynamics provide support for the tripartite model proposed by Kitajima and Butler.

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1. Introduction

The reactions in photosynthesis involve light absorption by specialized antenna pigments followed by efficient energy migration to a reaction center complex whereupon the excitation is utilized to drive the photosynthetic process. Fig. 1 illustrates how the harvesting is thought to occur. The dots represent chlorophyll *a* (Chl *a*) molecules and RC represents a reaction center. Not shown are accessory pigment molecules which assist in absorbing light. There are a variety of accessory pigment species [1] with essentially many overlapping absorptions that together span the visible spectrum. Once excited, the accessory pigment molecules very quickly (~ 1 ps) transfer their energy via a nonradiative dipole-dipole interaction to a Chl *a* molecule. The Chl *a* is first excited singlet state has a lower energy than the singlet states of the other antenna pigments and so the excitation cannot easily jump back. Possible, however, are further similar Förster transitions between neighboring Chl *a* molecules and consequently the excited state, or Frenkel exciton, hops a random walk through the Chl *a* antenna matrix until it reaches the reaction center complex whereupon it is quickly captured. The net result is that from initial pigment absorption to reaction center capture, although involving many hundreds of separate energy transfer steps, occurs in less than a femtosecond and has a quantum yield for photon utilization greater than 100%.

The hypothetical model of the antenna system of a plant shown in Fig. 1 is highly idealized. The actual situation is far more complicated and the exact organization of the antenna system is unknown. Photosynthesis in green plants takes place on a thin (10 nm) membrane called the thylakoid which is shaped like a flattened cylinder. The major constituent of the thylakoid is chlorophyll which exists in a variety of chlorophyll-protein complexes [1]. The various protein complexes, each composed of a dozen or so chlorophyll molecules (or accessory pigments) or other specialized photosynthetic centers (cytochromes, ferredoxins, etc.), are the building blocks of the photosynthetic apparatus. The dimensions of these building blocks are unfortunately too small to discern their precise organization using electron microscopy. The results rely on indirect information such as that obtained with picosecond spectroscopic techniques. With the highest magnification, repetitive structures, called quantasomes, measuring about 20 by 15 by 10 nm and arranged in a brick-like cobbles-stones can be seen on the thylakoid membranes. These quantasomes are often identified with the photosynthetic unit (~ 300), a hypothetical entity which contains all the functional material necessary to perform photosynthesis independently. There is evidence for and against the existence of such units [1,2]. If the units are independent, each would contain one reaction center

Fig. 1 Simplified representation of the antenna system of a plant. Light is absorbed by accessory pigments (not shown) and transferred to Chlorophyll a molecules (black dots) which funnel the excitation to a reaction center (RC)

moving its own antenna system as depicted in Fig. 1. This model is often referred to as the "paddle" model. An alternative model does not recognize individual PSI's and postulates a common antenna feeding all reaction centers. This model is often referred to as the "ladder" model. In this model, an exciton upon encountering a filled or closed reaction center would continue to migrate to another RC. This would not be possible in the paddle model where the quantum light eventually be lost to fluorescence. The question as to whether the ladder or paddle description is most appropriate is one which is still to be satisfactorily resolved in the literature.

An additional complication is introduced because in green plants there are thought to be at least two photosystems [2], PSI and PSII, which are in close proximity, each having their own distinct cooperative photochemistry. PSI, the smaller of the two units, upon red light absorption produces a strong reductant and a weak oxidant, whereas PSII, upon absorption of 'bluer' photons produces a strong oxidant and a weak reductant. Electron flow from the weak reductant to the weak oxidant is coupled to the conversion of adenosine diphosphate and inorganic phosphate to adenosine triphosphate (ATP). ATP assists the strong reductant to reduce CO_2 to a carbohydrate (CH_2O), and the strong oxidant oxidizes H_2O to O_2 . PSI and PSII are thought to be located on the outer and inner surfaces of the thylakoid, respectively.

The various techniques of picosecond spectroscopy can be readily employed to deduce information about the antenna apparatus of plants that is not presently obtainable by other means. One such scheme, see Fig. 1, would involve excitation by a picosecond laser pulse at an appropriate wavelength and simultaneous ultrafast streak camera observation of the fluorescence from various molecular species. The observed fluorescence would accurately probe the excited state

density of the species under observation. In particular, in green plants, the fluorescence decay from Chl *a* is predominantly determined by the "exciton" migration time to the reaction center, which effectively quenches the Chl *a* excited state population. This rate can in turn be utilized to test various models under consideration. Further information may be obtained by studying the additional quenching mechanisms that come into play at higher excitation intensities. For example, by flooding the antenna network shown in Fig. 1 with additional excitons, CAWILL et. al. [3,4] have shown that the excitons have a high probability of interacting with each other via a singlet-singlet fusion process. This process can be treated as a transition from a quantum state $S_1 + S_1$ to a shorter fluorescence decay times. Here S_1 is the first excited singlet state of Chl *a*, S_0 its ground state, and S_n higher excited states. The manner in which exciton interaction in vivo should strongly reflect the existence or non-existence of boundary conditions (as the model), provide information on the coupling of the two antennae, and also assist in obtaining values for various antenna fluorescence decay constants with which the fluorescence decay curve can be compared to determine which model best describes the coupling and organization of the antenna systems of green plants [5,6].

2. Experimental Setup

The experimental apparatus utilized in this experiment for the excitation of the sample and quantum yield measurement was described previously [7] and is similar to that shown in [8], which appears elsewhere in this volume. Briefly, a 1000 mW laser was filtered from above the sample by a heat blocked Nd:YAG laser, frequency shifted to 549 nm in a Raman cell, and then allowed to excite the sample, which in this experiment is a suspension of chlorophylls at 77 K. The sample was illuminated by directing the glow discharge from the laser cell through the aperture of a lens onto the sample area (see Fig. 1). A pinhole of the selected aperture of the lens image was placed in a converging lens, so that the laser energy was focused on the sample. The fluorescence was collected by a lens and focused onto the slit of an image camera (166 cm² × 25 cm) equipped with a cooled CCD. Appropriate narrow band interference filters were used to isolate the fluorescence for all interests. The fluorescence was collected with a silicon photodiode and analyzed with the displayed on a computer using a microcomputer. Stages could be accumulated on a microcomputer screen, then by evening exciton population effects and also constructed a graph of the signal to noise ratio. Because there is considerable variation from shot to shot in the area of the streak due to the electrical jitter, a reference point in time was established by calibrating a red-green pulse, which begins to be played on enter the streak, and a few hundred picoseconds before the arrival of the fluorescence. The zero time for the fluorescence onset and the excitation pulse has characteristics about the zero time position could be determined by examining the red light scattered off the sample. The fluorescence lifetime measurements, total chlorophyll fluorescence yields were obtained by detecting the fluorescence with a photodiode and a lock-in amplifier.

3. The Singlet-Singlet Fusion Process

CAWILL et. al. [3,4] proposed and demonstrated that Chl *a* singlet fusion processes occurred readily in photosynthetic organisms when excited with relatively low energy ps pulses. SHANK et. al. [9] and CAWILL et. al. [10] were able to fit early observations with a surprisingly simple formulation presented here. A Stern-Volmer rate equation which includes the effect of

exciton annihilation is given by

$$\frac{dn_s}{dt} = C I(t) - kn_s - \gamma n_s^2 \quad (1)$$

In (1), n_s is the population density of excited Chl *a* singlets, k is the reciprocal of the observed lifetime at low intensities, γ is the appropriate bimolecular rate constant, $I(t)$ is the laser intensity and C is a constant which includes the absorption cross section. If $I(t)$ is approximated by a delta-

$$n_s(t) = \frac{1}{-k + \sqrt{k^2 + \frac{4C}{n(0)} + \frac{4C}{k}}} e^{-kt} \quad (2)$$

In (2), $n(0)$ is the initial density after laser excitation. An expression for the total time integrated fluorescence yield $\Phi(t)$ can be obtained straightforwardly and is given by

$$\Phi(t) = \frac{\gamma}{k} \ln \left(1 + \frac{C I}{k} \right) \quad (3)$$

Here Φ is the yield at low I and $\Phi = \frac{\gamma}{k}$. This simple formalism has been shown to completely describe the exciton dynamics of mutants of the photosynthetic bacteria, *Rhodospseudomonas sphaeroides* [10]. However, because this model assumes no boundary conditions (i.e. lake model) and does not take into account the presence of two photosystems, it remained until the work described here to demonstrate the usefulness of this formalism in the case of green plants.

We should mention that to include the possibility of incorporating the particle model into the formalism would require the use of Poisson statistics and the expression for Φ would be drastically different from (3). Thus essentially it is straightforward to differentiate between the two cases.

4. Constructing an Appropriate Model of the Green Plant Antenna Apparatus

The most general model we might construct would consist of separate PSI and PSII reaction center complexes, each possessing its own antenna system. Although this is reasonable, there exists evidence which would seem to question this general model. For example, when the photosynthetic membranes of the higher plants are fractionated using detergents, several different chlorophyll-protein complexes can be identified. Ten to eighteen per cent of the total chlorophyll occurs in a type of protein-chlorophyll complex which is enriched with the P700 reaction centers of PSI [11]. Another 40-60% of the chlorophyll occurs as protein complexes containing equal amounts of chlorophyll *a* and *b*; these complexes have been designated as light-harvesting (LH) chlorophyll-protein complexes [11]. The LH pigment-protein complexes act as antenna, and it has been proposed that they can feed excitation energy to either of the two photosystems. A third type of chlorophyll-protein complex is believed to be closely associated with the photosystem II reaction centers [12]. Based on this tripartite organization of chlorophyll *in-vivo*, Butler and Kitajima have proposed that the low temperature fluorescence bands of chloroplasts at 685, 695 and 735 nm can be assigned to the LH, PSII and PSI chlorophyll-protein complexes respectively, [12,13]. Various fluorescence induction and quenching experiments at 77 K using cw sources have provided

some support for this tripartite fluorescence model [13-15].

We have therefore tested the validity of the tripartite model by examining the fluorescence from the 685, 695 and 735 bands at 77 K in spinach chloroplasts at various excitation intensities. The major portion of the experiments and conclusions presented in this section were previously published in two papers [5,6] and were performed in collaboration with R.E. GEACINTOV and S. L. SLENNBERG at N.Y.U., J. BRUION at the Centre d'Etudes Nucleaires de Saclay, and R. C. BYER at the Los Alamos Scientific Laboratory.

The results of our preliminary experiments are presented schematically in Fig. 2. The dots represent but a few of the hundreds of data points determined at the emission wavelength of 735 nm while the solid line summarizes numerous data points observed at 685 nm. Both curves are normalized by dividing by their respective low intensity quantum yield ϕ_0 . Surprisingly, both curves overlap. The functional roll-off is characteristic of singlet-singlet annihilation processes, and indeed the solid line in Fig. 2 is also exactly (2) with the appropriate rate constants. If we assume a stoichiometric ratio of 300 Chl *a* molecules to one reaction center when $X=1$ indicated in Fig. 2 represents one 530 nm photon absorbed per reaction center. Using this assumed absorption cross-section and the observed lifetime of PSII emission at low intensities (300 ps), (3) can be fit to our data with $k_2 = 5 \times 10^{-10} \text{ cm}^3/\text{sec}$.

If we assume that the 685 and 735 nm emission originates from different antenna pigment systems and that singlet-singlet annihilation occurs within both systems, it is difficult to explain why the two quenching curves in Fig. 2 are identical. The reasons against this are the following: (a) the lifetimes

Fig. 2 Relative fluorescence yield ϕ as a function of the incident intensity I_0 determined at the emission wavelength- of 685 and 735 nm (spectrum shown in insert)

of the singlet excitons are different in the two photosystems, the decay at 735 nm measured by us being 1.5 ns, while the decay time at 685 nm is 0.8 ns. A more efficient quenching at 735 nm is thus expected. (B) There is a greater pigment heterogeneity in the light harvesting pigments than within the PSI pigments and consequently one would expect different τ . (C) The distribution of energy and thus the density of singlet excitons may be different within the PSI and II light harvesting systems. All these factors should give rise to a difference in the quenching curves observed at 685 and 735 nm. No such differences are observed. It is highly unlikely that all of the above mentioned factors will fortuitously combine to cancel all these differences, and to produce identical quenching curves at 685 and 735 nm. If PSI and PSII are coupled to a common light harvesting system, as proposed in the tripartite model, and the exciton annihilation occurs in the LH system, then the data is consistent. Furthermore, the excellent fit of (3) to the data of Fig. 2 implies that the "lake" model is the appropriate description for this common light harvesting pool. A value for the exciton diffusion length (≈ 20 nm) can be derived [16] using the value of τ observed here and is consistent with a picture of the exciton ranging over several reaction centers (lake model).

The lifetime data is equally informative. Fig. 3 shows signal averaged emission data from PS II and I at an excitation intensity of 2×10^{14} photons/cm² and represents one of a series of such curves taken over a range of intensities. The data can be summarized:

- I. No difference was observed between the 685 nm and 695 nm emission, both displaying a 800 ps exponential decay at excitation intensities of 5×10^{13} photons/cm² or less and shorter nonexponential decays, consistent with (2) at higher intensities.
- II. The risetime of the 685 nm fluorescence is less than the resolution of our ps fluoroscope (20 ps).
- III. The decay time of the 735 nm emission was 1.5 ns and showed no variation with intensity over the range 5×10^{12} to 8×10^{15} photons/cm².
- IV. A risetime of 140 ± 50 ps (10-90%) was observed for the 735 nm emission

Fig.3 Time resolved fluorescence from spinach chloroplasts at a temperature of 77 K and an incident intensity of 2×10^{14} photons cm⁻². Emission at 690 nm rises promptly, following 530 nm excitation pulse, whereas emission at 735 nm rises much more slowly. (from Ref. [6])

at intensities below 10^{15} photons/cm² and rapidly decreased above that value.

The different dependence of the observed lifetimes of PS I and PS II emission with intensity despite the fact that their total time integrated fluorescence quantum efficiency quenching curves were identical further supports the picture of a tripartite organization with the exciton annihilation process occurring in the LH system. The observations I. and II. above indicate that PSII is strongly coupled (forwards and backwards) to the LH system. The rise and decay curves shown in Fig. 2 and the observed lifetime in IV. above indicate that PSI is coupled to LH with a rate constant $K = (140 \text{ ps})^{-1}$. The lack of variation of the 735 nm lifetime with intensity would imply little or no back reaction with the LH system. Also implied in the data is that the cross-section for PSI absorption must be $1 \text{ \AA}^2$ or less due to the observed lack of evidence of multiple excitation, even at intensities of 8×10^{15} photons/cm². This cross-section appears to be too small to represent Chl *a* emission from PSI. Recently, SATOH and BUTLER [17] proposed that the 735 nm fluorescence is due to the formation at low temperature of a Chl *a* species within PSI called C705. The proposed C705 species would have a cross-section of the appropriate size to account for our data. This and previous data presented in [17], we believe, confirms the assignment of the C705 species as the source of the 735 nm emission. Since C705 exists only in PSI, its emission provides an accurate probe of the exciton dynamics within PSI and does not affect our previous conclusions concerning the validity of the tripartite model.

There is yet an inconsistency in the data if we treat the LH, PSII and PSI aggregate as lumped systems with the following assumptions: 1) The LH and PSII are so closely coupled that they are indistinguishable and have a common fluorescence decay rate of $(800 \text{ ps})^{-1}$ 2) The rate of energy transfer from LH to PSI is $K = (140 \text{ ps})^{-1}$ and 3) PSI fluorescence decays at a rate $(1.5 \text{ ns})^{-1}$. The solution of the rate equations for such coupled systems predicts that the PSII fluorescence decay rate and PSI risetime should both equal $(K + 800 \text{ ps})^{-1} \sim 140 \text{ ps}$ at low intensities and $(800 \text{ ps})^{-1}$ at high intensities. This is not observed except at intensities greater than 10^{15} photons/cm². The difficulty probably arises because the LH aggregate is a distributed network of molecules and not a lumped element. However, by postulating two types of LH systems, LH(1) and LH(2), the data appears consistent once again. The two types of systems are coupled together in a common LH pool, but LH(1) is distinguishable in that its chlorophyll molecules are tightly coupled to the PSI pigment system with $\beta_{12} > \beta_{21}$. It is possible that LH(1) represents a relatively small fraction of the total light harvesting pigments, perhaps consisting of LH pigments which are in close proximity with PSI. LH(2) consists of chlorophyll molecules which are less tightly coupled to PSI (possibly because of a large physical separation) and which decay mainly by the rate constant, $\beta_{22} \sim (800 \text{ ps})^{-1}$. The fluorescence yield from this system is relatively higher than that of PSI. The 735 nm risetime is thus unaffected by exciton annihilation within LH(1) up to intensities of 10^{15} photons/cm² when the annihilation rate begins to compete with the rapid LH(1) to PSI transfer rate, K . Fig. 4 depicts schematically this modified tripartite fluorescence model.

5. Quenching of PSI Fluorescence by Triplet Exciton

Fig. 5 summarizes a recent experiment performed in collaboration with N. E. GEACINTOV and C. E. SWENBERG of N.Y.U. and K. WINN of Los Alamos [18]. Unlike previous experiments which were performed using single picosecond excitation pulses, here the excitation source, as shown in Fig. 5A, is a short train of

Fig. 4 Modified Tripartite Fluorescence Model (from Ref. [6])

Fig. 5 (A) Intensity profile of a typical pulse sequence. (B) Fluorescence (PSI) decay times measured at 77 K, 735 nm emission band. (C) $K[Q]$, quenching constant $\times$ quencher concentration within PSI calculated from the experimental data in (B). $\chi_{TS}[T]$ is a theoretical curve representing the predicted buildup of triplets, $[T]$, in the LH system $\times$ χ_{TS} , the singlet-triplet exciton annihilation rate constant. (from Ref. [18])

530 nm pulses, with a peak intensity of 5×10^{15} photons/cm² pulse. When isolated chloroplasts of spinach at 77 K are excited, it is found that the decay of the 735 nm fluorescence is initially 1.5 ns but fluorescence from later pulses display shorter exponential decays. The 1/e points of the observed decays are plotted in Fig. 5B. It can be seen that later pulses in the train produce successively shorter 735 nm fluorescence lifetimes. Presumably, a long lived intermediate state, most likely a triplet state, is created by intersystem

excimer-like dimers, formed by earlier pulses in the train. The triplets, in turn, quench the fluorescence via a singlet-triplet fusion process (i.e. $S_1 + T_1 \rightarrow S_0 + T_0$ or $S_0 + T_1 + \text{heat}$) and have an expected decay rate, $(\tau_1 + \tau_3)^{-1}$. Term Q is the population of the quencher and k the appropriate bimolecular rate constant. This effect partially accounts for the anomalously short fluorescence lifetime observed by early picosecond researchers when they excited chlorophylls with pulse trains. In Fig. 5C, the experimentally determined values of τ_1/τ_3 shown in Fig. 1B is plotted. Also plotted is a calculation predicting the expected value of τ_1/τ_3 [1] based on the anticipated buildup of T_1 and T_0 in the absence of quenching, and the decay rates of the T_1 and T_0 states.

The quenching rate constant k is calculated by assuming a value of τ_1/τ_3 experimentally determined for chlorophyll *a* in chloroform and also assumes a value of $\tau_1/\tau_3 = 6 \times 10^{-9}$ s, predicted by PAVLEN and ELLIX [19]. Considering the nature of the quenching, made in the calculation, there is good agreement between the absorption cross sections of T_1 and T_0 . If the magnitude of the intersystem crossing rate constant k_{ISC} is assumed to be different, this will affect the magnitude of the calculated quenching rate constant curve. We interpret the difference in the magnitude of the quenching rate constant as a consequence of triplet-triplet quenching, 50 ns in chlorophyll *a* in chloroform, as a consequence of triplet-triplet quenching, 50 ns in chlorophyll *a* in chloroform, as a consequence of triplet-triplet quenching, 50 ns in chlorophyll *a* in chloroform. This result appears to be consistent with previous studies performed by our group in organic solid photosynthetic envelopes, where it was observed that the diffusion rates of singlet excitons were two orders of magnitude greater than those of triplet excitons.

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